

The present disclosure also provides the use of a virus-specific immune cell or a pharmaceutical composition according to the present disclosure, in the manufacture of a medicament for treating or preventing a disease or condition characterised by an alloreactive immune response. In one aspect, the present disclosure refers to use of a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR in the manufacture of a medicament for treating or preventing a disease or condition caused or exacerbated by an alloreactive immune response, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM). In one example, the present disclosure refers to use of a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR in the manufacture of a medicament for use in treating or preventing a disease or condition characterised by an alloreactive immune response, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

The present disclosure also provides a method of treating or preventing a disease or condition characterised by an alloreactive immune response, comprising administering to a subject a therapeutically or prophylactically effective quantity of virus-specific immune cells or a pharmaceutical composition according to the present disclosure.

In some examples, the disease or condition characterised by an alloreactive immune response is a disease or condition associated with allotransplantation.

In some examples, the disease or condition is graft versus host disease (GVHD).

In some examples, the disease or condition is graft rejection.

In some examples, the method comprises administering a therapeutically or prophylactically effective quantity of the virus-specific immune cells or pharmaceutical composition to a donor subject for an allotransplant prior to harvesting the allotransplant.

In some examples, the method comprises administering a therapeutically or prophylactically effective quantity of the virus-specific immune cells or pharmaceutical composition to a recipient subject for an allotransplant.

In some examples, the method comprises contacting an allotransplant with a therapeutically or prophylactically effective quantity of the virus-specific immune cells or composition.

The present disclosure also provides a virus-specific immune cell or a pharmaceutical composition according to the present disclosure, for use in a method of treating or preventing a disease or condition by allotransplantation.

The present disclosure also provides the use of a virus-specific immune cell or a pharmaceutical composition according to the present disclosure, in the manufacture of a medicament for treating or preventing a disease or condition by allotransplantation. In one aspect, the present disclosure refers to use of a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR in the manufacture of a medicament for reducing or preventing an alloreactive immune response following allotransplantation, wherein the allotransplantation is to be performed for the treatment or prevention of a disease or condition treatable by allotransplantation, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a

signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM). In one example, the present disclosure refers to use of a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR in the manufacture of a medicament for use in treating or preventing a disease or condition by allotransplantation, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

The present disclosure also provides a method of treating or preventing a disease or condition by allotransplantation, comprising administering to a subject a therapeutically or prophylactically effective quantity of virus-specific immune cells or a pharmaceutical composition according to the present disclosure.

In some examples, the method comprises administering a therapeutically or prophylactically effective quantity of the virus-specific immune cells or pharmaceutical composition to a donor subject for an allotransplant prior to harvesting the allotransplant.

- 5 In some examples, the method comprises administering a therapeutically or prophylactically effective quantity of the virus-specific immune cells or pharmaceutical composition to a recipient subject for an allotransplant.

- 10 In some examples, the method comprises contacting an allotransplant with a therapeutically or prophylactically effective quantity of the virus-specific immune cells or composition.

In some examples, the allotransplantation comprises adoptive transfer of allogeneic immune cells.

- 15 In some examples, the disease or condition is a T cell dysfunctional disorder, a cancer or an infectious disease.

- The present disclosure also provides a method of killing alloreactive immune cells, comprising contacting alloreactive immune cells with a virus-specific immune cell or a pharmaceutical composition according to the present disclosure. In one aspect, the present disclosure refers to an *in vitro* method of killing
20 alloreactive immune cells, comprising contacting alloreactive immune cells *in vitro* with a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an
25 immunoreceptor tyrosine-based activation motif (ITAM).

- In one example, the present disclosure refers to a method of killing alloreactive immune cells, comprising contacting alloreactive immune cells *in vitro* with a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR for use in a method of treating or preventing a disease or condition characterised by an alloreactive immune response, wherein the CAR comprises: (i)
30 an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

35 **Description**

The inventors developed a CAR-modified virus specific T cell (CAR-VST) approach for the elimination of hematologic malignancies without causing GVHD, and avoiding allorejection.

- 40 The present disclosure provides a strategy to eliminate alloreactive T cells in order to protect allogeneic tissues including off-the-shelf cellular therapies from graft rejection, or to treat GVHD.

- CD30 has been identified as a marker of alloreactive T cells, and so the inventors targeted them by engineering therapeutic T cells to express a chimeric antigen receptor (CAR) directed against CD30 (CD30.CAR). CD30.CAR expressing VSTs can be employed in methods using allogeneic therapies, for
45 reducing alloreactive immune responses in the recipient subject.

Administering allogeneic T cells into HLA-mismatched recipients carries the risk of alloreactive immune responses such as GVHD since a proportion of the T cells will inherently possess alloreactivity. The inventors used virus specific T cells (VSTs) as the platform cells for expressing the CD30.CAR since they have been shown to rarely cause GVHD in allogeneic recipients, which is likely a result of their restricted TCR repertoire. In particular, Epstein-Barr Virus-specific T cells (EBVSTs) have been administered to more than 300 allogeneic recipients without any evidence of GVHD.

Claims:

1. Use of a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR in the manufacture of a medicament for treating or preventing a disease or condition caused or exacerbated by an alloreactive immune response, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).
2. The use according to claim 1, wherein the disease or condition caused or exacerbated by an alloreactive immune response is a disease or condition associated with allotransplantation.
3. The use according to claim 1 or claim 2, wherein the disease or condition is graft versus host disease (GVHD).
4. The use according to any one of claims 1 to 3, wherein the disease or condition is graft rejection.
5. The use according to any one of claims 1 to 4, wherein a therapeutically or prophylactically effective quantity of the virus-specific immune cells is to be administered to a donor subject for an allotransplant prior to harvesting the allotransplant.
6. The use according to any one of claims 1 to 5, wherein a therapeutically or prophylactically effective quantity of the virus-specific immune cells is to be administered to a recipient subject for an allotransplant.
7. The use according to any one of claims 1 to 6, wherein an allotransplant is to be contacted with a therapeutically or prophylactically effective quantity of the virus-specific immune cells.
8. Use of a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR in the manufacture of a medicament for reducing or preventing an alloreactive immune response following allotransplantation, wherein the allotransplantation is to be performed for the treatment or prevention of a disease or condition treatable by allotransplantation, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).
9. The use according to claim 8, wherein a therapeutically or prophylactically effective quantity of the virus-specific immune cells is to be administered to a donor subject for an allotransplant prior to harvesting the allotransplant.

10. The use according to claim 8 or claim 9, wherein a therapeutically or prophylactically effective quantity of the virus-specific immune cells is to be administered to a recipient subject for an allotransplant.
11. The use according to any one of claims 8 to 10, wherein an allotransplant is to be contacted with a therapeutically or prophylactically effective quantity of the virus-specific immune cells.
12. The use according to any one of claims 8 to 10, wherein the allotransplantation comprises adoptive transfer of allogeneic immune cells.
13. The use according to any one of claims 8 to 10, wherein the disease or condition treatable by allotransplantation is a T cell dysfunctional disorder, a cancer or an infectious disease.
14. An *in vitro* method of killing alloreactive immune cells, comprising contacting alloreactive immune cells *in vitro* with a virus-specific immune cell comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).
15. The use or the method according to any one of claims 1 to 14, wherein the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:26.
16. The use or the method according to any one of claims 1 to 15, wherein the transmembrane domain is derived from the transmembrane domain of CD28.
17. The use or the method according to any one of claims 1 to 16, wherein the transmembrane domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:20.
18. The use or the method according to any one of claims 1 to 17, wherein the antigen-binding domain comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:14, and an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:15.
19. The use or the method according to any one of claims 1 to 18, wherein the antigen-binding domain comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:18.
20. The use or the method according to any one of claims 1 to 19, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD3 ζ .
21. The use or the method according to any one of claims 1 to 20, wherein the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:25.

22. The use or the method according to any one of claims 1 to 21, wherein the CAR additionally comprises a hinge region provided between the antigen-binding domain and the transmembrane domain.
23. The use or the method according to claim 22, wherein the hinge region comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:33.
24. The use or the method according to any one of claims 1 to 23, wherein the CAR comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:35 or 36.
25. The use or the method according to any one of claims 1 to 24, wherein the virus-specific immune cell further comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30, or nucleic acid encoding a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30.
26. The use or the method according to any one of claims 1 to 25, wherein:
the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM) and an amino acid sequence derived from the intracellular domain of CD28; and
wherein the virus-specific immune cell further comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30, or nucleic acid encoding a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30.
27. The use or the method according to claim 25 or claim 26, wherein the virus-specific immune cell comprises more than one non-identical CAR, or nucleic acid encoding more than one non-identical CAR.
28. The use or the method according to any one of claims 25 to 27, wherein the target antigen other than CD30 is a cancer cell antigen.
29. The use or the method according to any one of claims 25 to 28, wherein the target antigen other than CD30 is selected from CD19, CD20, CD22, ROR1R, CD4, CD7, CD38, BCMA, Mesothelin, EGFR, GPC3, MUC1, HER2, GD2, CEA, EpCAM, LeY and PSCA.
30. The use or the method according to any one of claims 25 to 29, wherein the target antigen other than CD30 is CD19.
31. The use or the method according to any one of claims 1 to 30, wherein the virus-specific immune cell is a virus-specific T cell.
32. The uses or the method according to any one of claims 1 to 31, wherein the virus-specific immune cell is specific for Epstein-Barr virus (EBV).